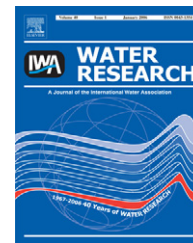


Available at [www.sciencedirect.com](http://www.sciencedirect.com)journal homepage: [www.elsevier.com/locate/watres](http://www.elsevier.com/locate/watres)

# Occurrence and fate of endocrine disrupters in Greek sewage treatment plants

Athanasios S. Stasinakis<sup>a,\*</sup>, Georgia Gatidou<sup>a,b</sup>, Daniel Mamais<sup>c</sup>, Nikolaos S. Thomaidis<sup>b</sup>, Themistokles D. Lekkas<sup>a</sup>

<sup>a</sup>Water and Air Quality Laboratory, Department of Environment, University of the Aegean, University Hill, Mytilene 81 100, Greece

<sup>b</sup>Laboratory of Analytical Chemistry, Department of Chemistry, National and Kapodistrian University of Athens, Zografou, Athens 157 71, Greece

<sup>c</sup>Faculty of Civil Engineering, Department of Water Resources, National Technical University of Athens, 5 Iroon Polytechniou Str., Zografou, Athens 15773, Greece

## ARTICLE INFO

### Article history:

Received 5 June 2007

Received in revised form

17 September 2007

Accepted 6 November 2007

Available online 17 November 2007

### Keywords:

EDCs

Wastewater

Presence

Removal

Sewage sludge

Risk assessment

## ABSTRACT

The occurrence of five endocrine-disrupting compounds (EDCs), namely 4-*n*-nonylphenol (4-*n*-NP), nonylphenol monoethoxylate (NP1EO), nonylphenol diethoxylate (NP2EO), triclosan (TCS) and bisphenol A (BPA), was assessed in the raw, treated wastewater and sewage sludge of eight sewage treatment plants (STPs) in Greece. The analytes were extracted by solid-phase extraction (dissolved phase) or sonication (solid phase). Qualitative and quantitative analyses were performed by gas chromatography-mass spectrometry (GC-MS). The average concentrations in the raw and treated wastewater ranged from 0.23 (4-*n*-NP) to 5.76 µg L<sup>-1</sup> (NP1EO) and from 0.15 (BPA) to 1.84 µg L<sup>-1</sup> (NP2EO), respectively. A great part of the detected EDCs was sorbed on suspended solids. In sewage sludge, the average concentrations ranged between 0.17 (4-*n*-NP) and 12.3 µg g<sup>-1</sup> dw (NP1EO). Analysis of daily mass flows in STP of Athens showed that, with the exception of 4-*n*-NP, all other EDCs were significantly removed (>85%) during wastewater treatment. Regarding the fate of these compounds, a significant part ranging from 45% (for TCS) to more than 70% (for NP1EO, NP2EO and BPA) was transformed by abiotic or biotic mechanisms, while the rest was accumulated in sewage sludge or disposed to the environment via the effluents. Calculation of risk quotients showed the existence of possible threat due to the presence of certain EDCs in treated wastewater and sludge.

© 2007 Elsevier Ltd. All rights reserved.

## 1. Introduction

Several synthetic organic compounds that have been classified as endocrine disruptors (EDCs) are commonly detected in municipal wastewater. Among these, nonylphenol (NP), NP ethoxylates (NP<sub>*n*</sub>EOs, where *n* indicates the number of ethoxy units), bisphenol A (BPA) and triclosan (TCS) present significant research interest due to their extensive use and their physicochemical and toxicological properties.

NP<sub>*n*</sub>EOs are an important group of non-ionic surfactants that are widely used in many commercial and household functions, including detergents, cosmetic products and textiles (Birkett and Lester, 2003). It has been proved that biotransformation of long-chain NP<sub>*n*</sub>EOs, which occurs in sewer system, results in accumulation of shorter-chain metabolic intermediates including NP, NP monoethoxylate (NP1EO) and NP diethoxylate (NP2EO) (Ahel et al., 1994). These compounds have been classified as EDCs by several organizations

\*Corresponding author. Tel.: +30 22510 36257; fax: +30 22510 36246.

E-mail address: [astan@env.aegean.gr](mailto:astan@env.aegean.gr) (A.S. Stasinakis).

0043-1354/\$ - see front matter © 2007 Elsevier Ltd. All rights reserved.

doi:10.1016/j.watres.2007.11.003

(Birkett and Lester, 2003), while NP has been listed as a priority substance in the Water Framework Directive (EU, 2001). Moreover, the European Union, in an attempt to set some limit values for trace organic contaminants in sludge, proposed in a Working Document a limit value of  $50 \mu\text{g g}^{-1} \text{dw}$  for NPEs (sum of NP, NP1EO and NP2EO) (EU, 2000). Regarding NP, this compound is a mixture of different branched and linear-chain isomers (*ortho*-, *meta*- or *para*-), with the most common ring isomers being the *para* isomers (4-NPs). Recent studies have shown that 4-*n*-NP is a strong estrogenic isomer (Vetillard and Bailhache, 2006) that is usually used as the representative compound of this category (Ying et al., 2003; Calafat et al., 2005).

BPA is widely used for the production of flame retardants, polycarbonate and epoxy resins. These products are used in food and drink packaging, as additives in thermal paper and in dental fillings. It has been shown that BPA possesses weakly estrogenic activity, as well as antiandrogenic activity (Birkett and Lester, 2003). TCS is a broad-spectrum antimicrobial and preservative agent that is widely used in personal-care products. In Europe, approximately 350 t of TCS are produced annually for commercial applications (Singer et al., 2002), while in USA, more than  $300 \text{ t yr}^{-1}$  of TCS are estimated to be disposed into wastewater (Halden and Paull, 2005). TCS may act as an endocrine disruptor via activation of the human pregnane X receptor (Jacobs et al., 2005).

So far, the occurrence of these EDCs in sewage treatment plants (STPs) has been well documented through several studies around the world (Mc Avoy et al., 2002; Nakada et al., 2006; Vogelsang et al., 2006). However, there are few data for the Mediterranean area (Gomez et al., 2007; Gonzalez et al., 2007), while no data are available for Greek STPs. In addition to the above, despite the existence of several studies investigating the presence of these EDCs in wastewater, most of them have been focused exclusively on aqueous-phase concentrations. Particulate matter is often not included in wastewater analysis and concentrations of these compounds in sludge are rarely determined (Heidler and Halden, 2007), probably due to the difficulty in accurately detecting and quantifying EDCs in this challenging analytical matrix. As a result, systematic data on balances of these EDCs in STPs are missing, and the environmental load of these compounds is still not adequately documented. On the other hand, due to the relatively high octanol–water partition coefficients of these compounds (Birkett and Lester, 2003; Heidler and Halden, 2007), it is reasonable to expect that a significant part of these EDCs is sorbed on the suspended solids or accumulated in biosolids. Moreover, based on the fact that the quantity of sludge generated in European STPs has been greatly increased in the recent years due to the implementation of the urban wastewater treatment Directive 91/271/EEC, one can assume that a substantial portion of EDCs is transferred to European soil.

To investigate the occurrence of EDCs in Greece, wastewater (raw and treated) and sewage sludge samples were collected during three sampling campaigns in 2006 from eight STPs, differing with respect to their size and operational conditions. An integrated analytical method was applied for the simultaneous determination of 4-*n*-NP, NP1EO, NP2EO, BPA and TCS in wastewater (dissolved and particulate phase) and sewage sludge, and the detected concentration levels

were compared with those from the literature, while the possible threat due to disposal of these compounds in the environment was estimated and discussed. Daily mass flows normalized to inhabitants were also calculated for each STP and distribution of EDCs between dissolved and particulate phase was determined. Moreover, a mass balance was performed for the greatest STP examined in this study (Athens, Greece) to investigate the fate of EDCs during wastewater treatment and to estimate the role of degradation and sorption on their removal.

## 2. Materials and methods

### 2.1. Sampling and STPs surveyed

Wastewater samples were collected from six STPs (STP A–F) in Greece (Figure S1). Information on the individual plants and their operational parameters are summarized in Table 1. The average sewage flows in the STPs included in this study varied from 30 to  $650\,000 \text{ m}^3 \text{ d}^{-1}$ , while all STPs were equipped with secondary treatment (activated sludge process).

Three sampling campaigns were performed in March, June and September 2006. During these months precipitation is low, so dilution of the sewage influent is minimal and the concentrations of any compound found would therefore represent a ‘worst case scenario’. Twenty-four-hour composite samples of sewage influents and secondary effluents were taken on 3 consecutive days from STP A in each sampling campaign, while grab samples were taken from STP B with a similar frequency. Grab influent and secondary effluent samples were also collected once in each sampling campaign from the other STPs. During this study, BOD removal was higher than 95% in all STPs, except for STP E, where operating problems had been noticed due to insufficient capacity of the mechanical aerators.

Grab sludge samples were additionally collected in the aforementioned sampling campaigns from STPs A, B and C. During the last sampling campaign, three samples were also taken in consecutive days from STPs serving the cities of Nafplio (STP G) and Herakleio (STP H) (Figure S1). All sludge samples were either dewatered anaerobic digested sludge (STPs A, C and H) or dewatered secondary sludge (STPs B and G) (Table 1).

Wastewater samples were collected in 2 L pre-cleaned amber glass bottles, while sludge samples were collected in glass jars. All samples were transported to the laboratory in a cooler. Wastewater samples were filtered through pre-ashed glass-fiber filters (GF/F, Whatman, UK) and stored in the dark at  $4^\circ\text{C}$  until solid-phase extraction (SPE) (normally 24 h after filtration). For the determination of EDCs in the particulate phase, filters were oven dried until constant weight and stored at  $-18^\circ\text{C}$  until their analysis. Sewage sludge samples were oven dried at  $40^\circ\text{C}$  (Gatidou et al., 2007), grinded using a mortar and pestle and stored at  $-18^\circ\text{C}$  until their analysis. All samples were analyzed within a period of 5 days.

### 2.2. Chemical and standards

Analytical standards of 4-*n*-NP (99.5%), NP1EO (99%) and NP2EO (99%) were supplied by Dr. Ehrenstorfer-Schafers

Table 1 – Operating data of the sewage treatment plants included in this study

Plant	STP ID	Treatment processes	Population served	Average flow (m <sup>3</sup> /day)	Contribution from industry (%)	Average sludge production (kg DS/day)	HRT <sup>a</sup> (h)	SRT <sup>b</sup> (d)
Athens	A	S+G+Sed+AS(BNR)+Sed+AD+Dew	3 500 000	650 000	20	180 000	9	8
Mytilene	B	S+G+AS(BNR)+Sed+Chl+Dew	24 000	4500	<5	1100	23	18
Chalkida	C	S+G+Sed+AS(BNR)+Sed+UVdis+AD+Dew	50 000	10 000	10	2500	8	8
University	D	S+AS(N)+Sed+Chl	1000	30	0	- <sup>c</sup>	35	23
Hospital	E	S+AS(N)+Sed+Chl	600	120	0	-	22	-
Airport	F	S+AS(N)+Sed	1200	50	0	-	32	25
Nafplion	G	S+G+AS(BNR)+Sed+Chl+Dew	40 000	10 000	<5	1800	-	-
Herakleion	H	S+G+Sed+AS(BNR)+Sed+AD+Dew	100 000	25 000	<5	5000	-	-

Abbreviations: S = screening, G = grit removal, Sed = sedimentation, AS = activated sludge, BNR = biological nutrient removal, N = nitrification, Chl = chlorination, UVdis = UV disinfection, AD = anaerobic digestion, Dew = mechanical dewatering.

<sup>a</sup> Hydraulic retention time in aeration tank.

<sup>b</sup> Sludge residence time in activated sludge process.

<sup>c</sup> Not known.

(Germany). BPA (>97%) was purchased from Buchs (Switzerland), whereas TCS (>97%) and deuterated BPA (BPA-16), used as internal standard, were purchased from Fluka (Germany). Stock solutions of individual compounds were prepared in methanol at 1000 mg L<sup>-1</sup> and kept at -18°C. The stock solutions were used to prepare regularly working standard solutions for calibration.

### 2.3. Sample preparation and analysis

Analytical methods for the determination of EDCs in wastewater (dissolved and particulate phase) and sewage sludge samples were developed and optimized by the authors (Gatidou et al., 2007) and they are briefly illustrated in Fig. 1. The developed procedure included either SPE (liquid samples) or sonication (solid samples), while for the qualitative and quantitative analyses a Hewlett Packard Gas Chromatograph 5890 Series II connected to a Hewlett Packard Mass Spectrometer HP5971 MSD was used (USA). The separation of EDCs was achieved using a DB5MS capillary column (60 m) with a film thickness of 0.25 µm and an internal diameter of 0.32 mm (Supelco, USA).

Detailed information about the quality parameters of the analytical methods have been reported by Gatidou et al. (2007). In brief, these analytical methods presented satisfactory precision, with relative standard deviations less than 12% for all the tested compounds. Satisfactory recoveries were obtained for all the compounds, ranging from 57% to 89% for aqueous phase and from 78% to 104% for solid phase,

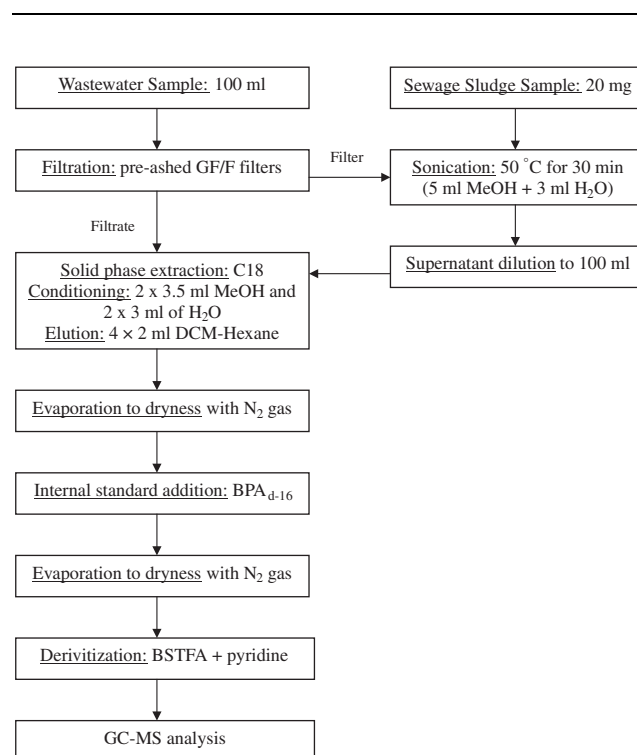


Fig. 1 – Schematic diagram of the applied analytical method for the determination of the target EDCs in wastewater and sludge samples.

except for 4-*n*-NP, where recoveries of 33% and 55% in water and sludge samples were obtained, respectively (Gatidou et al., 2007). For this reason, recovery correction was made for 4-*n*-NP (Nakada et al., 2006), by dividing the observed concentrations by recovery rates. Limits of detection (LODs) of the target compounds varied from 0.03 (4-*n*-NP) to 0.41  $\mu\text{g L}^{-1}$  (NP2EO) and from 0.04 (4-*n*-NP) to 0.96  $\mu\text{g g}^{-1}$  dw (NP2EO) for liquid and solid samples, respectively (Gatidou et al., 2007). Whenever the influent or effluent concentrations were below the LOD, a concentration equal to half of the detection limit was used for the calculations (Chandrinou et al., 2007). On the basis of the volume of the samples filtered, the volume-basis concentrations (i.e.,  $\mu\text{g L}^{-1}$ ) were calculated for particulate EDCs. For the determination of total EDC concentrations in wastewater, the sum of dissolved and particulate concentration was used.

#### 2.4. Calculations

For sewage treatment plant A, the degree of removal obtained was calculated from the total (sum of dissolved and particulate) analyte concentration in raw sewage water ( $C_{\text{inf}}$ ) and final effluent ( $C_{\text{eff}}$ ) according to Eq. 1:

$$\text{Removal (\%)} = \frac{C_{\text{inf}} - C_{\text{eff}}}{C_{\text{inf}}} \times 100. \quad (1)$$

Moreover, the mass load of EDCs that was lost due to the sum of all transformation processes ( $W_{\text{lost}}$ ) was calculated according to Eq. 2 (Heidler and Halden, 2007):

$$W_{\text{lost}} = (Q_{\text{inf}} \times C_{\text{inf}}) - (Q_{\text{eff}} \times C_{\text{eff}}) - W_{\text{sludge}}, \quad (2)$$

where  $Q_{\text{inf}}$  and  $Q_{\text{eff}}$  are the flow rates of influents ( $\text{m}^3 \text{d}^{-1}$ ) and effluents ( $\text{m}^3 \text{d}^{-1}$ ), respectively, while  $W_{\text{sludge}}$  is the mass output of EDCs in dewatered digested sludge ( $\text{mg d}^{-1}$ ).

Mass flows, in milligrams per day, as well as mass flows normalized to inhabitants served at each STP, in milligrams per 1000 inhabitants per day, of individual EDCs were calculated from concentrations of each sampling occasion in each matrix, using the corresponding mean daily sewage volume and the corresponding mean daily sludge production (Table 1).

### 3. Results and discussion

#### 3.1. EDCs occurrence in the influent and effluent wastewater

During the three sampling campaigns, altogether 30 samples were collected from the influents and effluents of six different STPs. The data obtained from all the analyzed samples are presented in Table 2 and Table S1. Regarding the untreated sewage, TCS, NP1EO and NP2EO were detected in all samples, whereas 4-*n*-NP and BPA were found in 25 and 23 samples, respectively (Table 2). The highest value of the means and the maximum concentration were 16.4 (in STP E) and 23.9  $\mu\text{g L}^{-1}$  (in STP A), respectively, and these were due to TCS (Table S1). Contrary to TCS, BPA mean concentrations did not exceed 1.25  $\mu\text{g L}^{-1}$  (STP C). Regarding NPs, the maximum mean concentrations were 13.5 (STP A) and 9.34  $\mu\text{g L}^{-1}$  (STP C) for NP1EO and NP2EO, respectively. On the other hand, concentrations of 4-*n*-NP were significantly lower (Table 2, Table S1), probably due to the fact that this compound is one of the isomers that constitute NP. Based on daily loading and population served by each plant, the population-corrected loads were calculated (Table S2). STPs A, C and E presented the highest average loads for most of the EDCs (NP1EO, TCS and NP2EO), possibly due to the fact that STPs A and B co-treat industrial wastewater, while STP E treats hospital's wastewater (Table 1). In these STPs, influent average daily loads ranging up to 2504, 3285 and 1869 mg per 1000 inhabitants were calculated for NP1EO, TCS and NP2EO, respectively (Table S2). In the literature, there are few data available regarding EDCs loading in STPs. In a previous study, Mc Avoy et al. (2002) reported a TCS usage rate of 3000–5000 mg per 1000 inhabitants per day. Moreover, Heidler and Halden (2007) reported a TCS per-capita loading ranging from 390 to 1430  $\text{mg yr}^{-1}$ , which amounts to 1068–3918 mg TCS per 1000 inhabitants per day.

EDCs were not totally eliminated by the applied treatment processes and, as a result, were detected in most effluent samples (Table 2). In treated wastewater, TCS was the dominating compound and was found in 25 samples, possibly due to its high concentrations in untreated sewage and its low

**Table 2 – Occurrence of the target EDCs in the influents and effluents (in  $\mu\text{g L}^{-1}$ ) of Greek sewage treatment plants included in this study (six plants,  $n = 30$ )**

Substance	Influent						Effluent				
	LOD	[N] > LOD <sup>a</sup>	Mean	Median	Min	Max	[N] > LOD	Mean	Median	Min	Max
4- <i>n</i> -NP	0.03	25	0.23	0.17	<0.03	1.04	24	0.18	0.11	<0.03	0.90
NP1EO	0.34	30	5.76	3.30	0.35	20.8	15	0.89	0.17	<0.34	6.89
TCS	0.13	30	4.90	1.37	0.17	23.9	25	1.10	0.43	<0.13	6.88
BPA	0.14	23	0.73	0.68	<0.14	2.14	12	0.15	0.07	<0.14	1.10
NP2EO	0.41	30	3.99	2.38	0.71	13.4	15	1.84	0.20	<0.41	17.4

<sup>a</sup> [N] > LOD: number of samples with concentrations higher than the limit of detection (LOD) of the method.



LOD. However, among the target compounds, the highest mean and maximum concentrations were found for NP2EO in STP E (12.9 and 17.4  $\mu\text{g L}^{-1}$ , respectively), while lower NP2EO concentrations were detected in other STPs (Table S1). Regarding the other target compounds, maximum concentrations up to 0.90, 6.89, 6.88 and 1.10  $\mu\text{g L}^{-1}$  were detected for 4-n-NP, NP1EO, TCS and BPA, respectively (Table 2, Table S1).

Investigation of EDCs' distribution between particulate and dissolved phase showed that a significant part of these compounds accumulated on the suspended solids. In influent wastewater, almost 45–50% of the target compounds were found in the particulate phase, while in treated wastewater this fraction ranged between 18% and 35% (Fig. 2). The lower percent of particle-bound EDCs obtained in effluent wastewater was probably due to the fact that most of the particulate matter had been removed during secondary sedimentation and as a result low suspended solids concentrations were determined in the effluents (2–22  $\text{mg L}^{-1}$ ). So far, few studies have focused on EDCs' partitioning between the dissolved and particulate phase. Isobe et al. (2001) found that  $33 \pm 21\%$  of NP was in the particulate phase in secondary effluents. Moreover, similar percentages of distribution in particulate matter have been reported for NP, NP1EO, NP2EO and BPA in river water (Patrolecco et al., 2006). These observations indicate that employing advanced treatment processes for suspended total removal (e.g. filtration) could probably result in a further decrease in EDCs' loading to recipient water bodies.

For most of the cases, the concentrations of EDCs detected in this study fall into the range reported in the literature. An exception was noticed for TCS in effluent wastewater, where in few cases (Table S1, STP A, C and E) its concentration was higher than those previously reported in the literature. Specifically, TCS concentrations ranging from 0.39 to 26.8  $\mu\text{g L}^{-1}$  and up to 2.7  $\mu\text{g L}^{-1}$  have been detected in influent and effluent wastewater, respectively (Mc Avoy et al., 2002; Waltman et al., 2006; Gomez et al., 2007). Monitoring studies

in STPs of Austria and Spain have shown BPA concentration levels ranging up to 3.4 and 1.53  $\mu\text{g L}^{-1}$  in influent and effluent wastewater, respectively (Clara et al., 2005; Gomez et al., 2007). Regarding NPs, concentrations of NP, NP1EO and NP2EO up to 58, 150 and 230  $\mu\text{g L}^{-1}$ , respectively, have been reported for influent wastewater (Planas et al., 2002; Fauser et al., 2003; Gonzalez et al., 2007), while concentrations up to 4, 53 and 29  $\mu\text{g L}^{-1}$  have been detected in effluent wastewater (Vogelsang et al., 2006; Gonzalez et al., 2007).

Concentrations of EDCs detected in influent wastewater were significantly lower than those reported in the literature for inhibiting activated sludge process. Federle et al. (2002) reported no adverse effects on the activated sludge process when TCS was incrementally increased from 0.04 to 2  $\text{mg L}^{-1}$  in a lab-scale activated sludge system. Moreover, in a recent study, Stasinakis et al. (2007) reported that TCS concentrations as low as 500  $\mu\text{g L}^{-1}$  slightly inhibited nitrification capacity, while there was no effect on COD removal. Finally, toxicity experiments with ammonia-uptake rate and specific oxygen-uptake rate showed that 50  $\text{mg L}^{-1}$  4-n-NP slightly inhibited autotrophic activated sludge microorganisms, while there was no effect on heterotrophic microorganisms (Stasinakis et al., 2006).

Regarding the effects of target EDCs in the aquatic environment, measured environmental concentrations (MECs) of EDCs in influent and effluent wastewater were used together with predicted no observed effect concentrations (PNECs) obtained from peer-reviewed literature, to calculate risk quotients expressed as MEC/PNEC ratios (Lindberg et al., 2007). According to the literature, if the exposure concentration exceeds the effect concentration ( $\text{MEC} > \text{PNEC}$ ), then an ecological risk is suspected (Lindberg et al., 2007; Ying and Kookana, 2007). However, it should be mentioned that the actual ecological risk should be lower than the one estimated due to effects such as dilution of effluent wastewater in the recipient water bodies. In influent wastewater, ratios above 1 were obtained for all the target

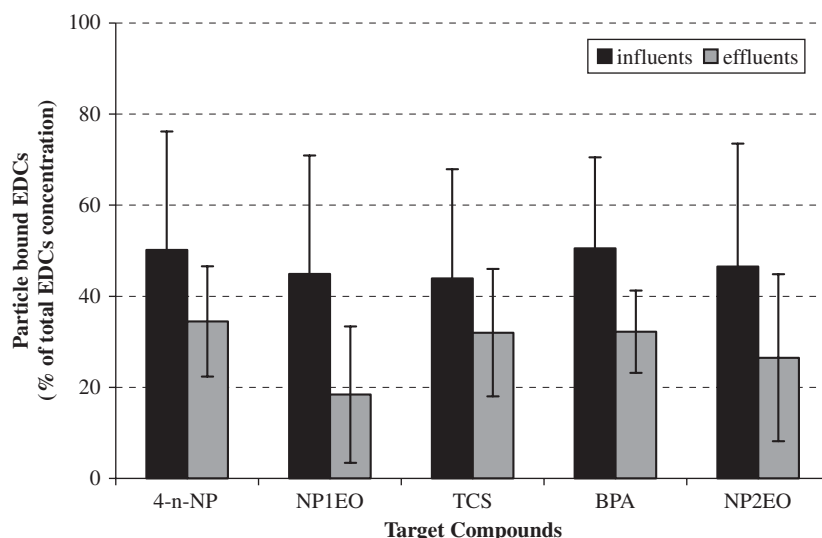


Fig. 2 – Fraction of EDCs bound to particulate matter in influent and effluent wastewater ( $n = 30$ ).

**Table 3 – Predicted no observed effect concentrations (PNECs) and ratios of measured environmental concentrations (MECs) to PNECs of EDCs detected in influent and effluent wastewater throughout Greece**

Substance	PNEC ( $\mu\text{g L}^{-1}$ )	MEC/PNEC											
		STPA <sub>in</sub>	STPA <sub>out</sub>	STPB <sub>in</sub>	STPB <sub>out</sub>	STPC <sub>in</sub>	STPC <sub>out</sub>	STPD <sub>in</sub>	STPD <sub>out</sub>	STPE <sub>in</sub>	STPE <sub>out</sub>	STPF <sub>in</sub>	STPF <sub>out</sub>
4-n-NP	0.33 <sup>a</sup>	3.2	1.3	1.2	2.7	0.9	1.9	0.9	0.2	0.6	0.2	1.5	1.3
NP1EO	0.11 <sup>b</sup>	189	7.0	47	5.9	113	28	17	1.5	40	63	76	22
TCS	0.05 <sup>c</sup>	478	92	46	23	390	121	20	8.8	344	138	66	30
BPA	1.0 <sup>d</sup>	1.3	0.3	2.1	0.3	2.1	1.1	1.7	0.1	1.0	0.2	1.1	0.2
NP2EO	0.11 <sup>b</sup>	99	8.5	66	18	122	1.9	24	9.2	101	158	10	1.9

Maximum concentrations detected in each STP were used, whereas when MECs were not available, values equal to half LOD were used.

<sup>a</sup> European Commission (2002).

<sup>b</sup> Fenner et al. (2002).

<sup>c</sup> Ying and Kookana (2007).

<sup>d</sup> Fromme et al. (2002).

compounds in most STPs, indicating the risk for environmental threat in case the raw wastewater is directly discharged into the environment (Table 3). After wastewater treatment, risk quotient values higher than 1 were still obtained for NP1EO, TCS and NP2EO in all STP effluents, while values higher than 100 were obtained for TCS in STPs C and E and for NP2EO in STP E. On the other hand, a lower risk for the aquatic environment was estimated for 4-n-NP and BPA, whereas sporadically measured concentrations were higher than PNEC.

### 3.2. EDCs occurrence in sewage sludge

During this study, altogether 27 sludge samples were collected from five STPs of different size and operating principles. The data obtained from all the analyzed samples are presented in Table 4 and Figure S2. In all samples, NP1EO and TCS were detected above their LOD, whereas 4-n-NP, NP2EO and BPA were found in 26, 13 and 25 samples, respectively.

Concentrations of TCS as high as  $9.85 \mu\text{g g}^{-1}\text{dw}$  were detected (STP H), while, so far, concentrations ranging between 0.09 and  $55 \mu\text{g g}^{-1}\text{dw}$  have been reported in the literature (Heidler and Halden, 2007; Ying and Kookana, 2007). In the present study, the highest TCS concentrations were detected in STPs A, C and H (Figure S2). These concentrations could be attributed to the high TCS input load observed in these STPs (see Table S1), as well as to the presence of primary sedimentation in these treatment plants (Table 1). Previous studies have shown that a significant part of TCS is removed by primary sludge, being unavailable for microbial degradation in activated sludge process. Specifically, Mc Avoy et al. (2002) reported a TCS removal by primary treatment up to 48% in STPs in USA.

So far, few terrestrial toxicity data are available in the literature for TCS. In a recent study, Ying and Kookana (2007) calculated a PNEC value of  $0.096 \mu\text{g TCS kg}^{-1}\text{soil}$ . Assuming that sludge with the highest TCS concentration detected in the present study ( $9.85 \mu\text{g g}^{-1}\text{dw}$ ) was applied to soil (bulk density of  $1.3 \text{ kg L}^{-1}$ ) at a typical rate of  $10 \text{ t ha}^{-1}$  and tilled to 10 cm, a predicted effective concentration (PEC) of  $0.08 \mu\text{g g}^{-1}$  (dilution factor: 130) would be approximately calculated in a well-mixed soil. Based on the aforementioned PEC and PNEC values, the risk quotient is calculated to be 830, which is considerably more than 1. According to the above, a possible threat due to sludge disposal in soil is expected. This threat could be more severe keeping in mind that incomplete soil and sludge mixing could cause higher TCS-localized concentrations. However, more information on TCS toxicity to terrestrial microorganisms and its fate in the soil environment is needed in order to conduct a more realistic environmental risk assessment.

Contrary to TCS, BPA concentrations in sewage sludge did not exceed  $1.75 \mu\text{g g}^{-1}\text{dw}$  (Table 4) and there was no difference between different STPs (Figure S2). To the best of our knowledge, almost similar BPA concentration levels have been reported in the literature (Bolz et al., 2001). So far, there are no studies investigating BPA toxicity in soil. However, in a previous study investigating its fate in soil, Fent et al. (2003) estimated a half-life of less than 3 days and concluded that

**Table 4 – Occurrence of the target EDCs in the sewage sludge (in  $\mu\text{g g}^{-1}\text{ dw}$ ) of Greek sewage treatment plants included in this study (five STPs,  $n = 27$ )**

Substance	LOD	sludge				
		[N] > LOD <sup>a</sup>	Mean	Median	Min	Max
4-n-NP	0.04	26	0.17	0.15	<0.04	0.45
NP1EO	0.49	27	12.3	4.88	1.01	41.3
TCS	0.15	27	3.21	2.71	0.19	9.85
BPA	0.56	13	0.53	0.28	<0.56	1.75
NP2EO	0.96	25	6.14	3.70	<0.96	24.7

<sup>a</sup> [N] > LOD: Number of samples with concentrations higher than the limit of detection (LOD) of the method.

this compound is not expected to be stable and bioavailable after disposal to soil.

Regarding NPs, concentrations of 4-n-NP, NP1EO and NP2EO up to 0.45, 41.3 and 24.7  $\mu\text{g g}^{-1}\text{ dw}$  were detected, respectively (Table 4). Concentrations of 4-n-NP were similar in all STPs, whereas the highest mean concentrations of NP1EO were detected in STPs A, C and H (Figure S2). Moreover, the highest mean NP2EO concentration was detected in STP H (Figure S2). It should be mentioned that the aforementioned STPs include primary sedimentation and anaerobic digestion. The highest NPs concentrations detected in these STPs could be explained by the sorption of these compounds in primary sludge (Planas et al., 2002), as well as their further formation as metabolites from other nonylphenolic precursors during anaerobic digestion (Ahel et al., 1994). In all cases, NPE concentrations detected in the present study were lower than the limit fixed in the third draft of the EU Sludge Directive (50  $\mu\text{g g}^{-1}\text{ dw}$ ) (EU, 2000). The highest concentrations of NPEs were detected in STP C (49.2  $\mu\text{g g}^{-1}\text{ dw}$ ) and STP H (49.2  $\mu\text{g g}^{-1}\text{ dw}$ ). However, it should be mentioned that in this study 4-n-NP was used during analysis. It is possible that if technical mixture of NP had been used, NPEs concentrations exceeding the European limit could have been calculated in a few cases.

So far, little research has been done to examine NPs' effects on terrestrial biota and most of the reported data are controversial. In a recent study, Roberts et al. (2006) reported that NP had no negative effect on soil respiration or plant growth unless present at extremely high concentrations (>10000  $\mu\text{g NP g}^{-1}\text{ soil}$ ). On the other hand, Environment Canada (2000) recommended a PNEC for terrestrial risk of 0.34  $\mu\text{g g}^{-1}$ . Due to the limited toxicological data available for the other nonylphenolic compounds, a similar value for terrestrial risk was also assumed for NP1EO and NP2EO in the present study. Using a sludge disposal rate equal to that reported above (dilution factor of 130), PEC values of 0.003, 0.32 and 0.19  $\mu\text{g g}^{-1}$  were approximately estimated for 4-n-NP, NP1EO and NP2EO, respectively, in a well-mixed soil. According to the aforementioned values, a risk quotient lower than 1 was calculated for NPs, and as a result no significant threat due to sludge application in soil is expected.

### 3.3. EDCs' removal efficiency and fate in STP A

To determine percentage EDCs removal and to investigate their fate during wastewater treatment, Eqs. (1) and (2) were

used and daily masses of EDCs in influents, effluents and biosolids were calculated for STP A, where composite samples were taken during this study.

With the exception of 4-n-NP, where in several sampling occasions effluent concentrations were higher than influent concentrations, resulting in negative mean percentage removal (−9%), apparent removal efficiency was noticed for the other EDCs. The highest removal efficiency was calculated for NP1EO (98 ± 1%), whereas slightly lower removal efficiencies were observed for TCS (91 ± 6%), BPA (87 ± 7%) and NP2EO (91 ± 7%). So far, similar percentages of TCS and BPA removal have been reported in the literature (Nakada et al., 2006; Gomez et al., 2007; Ying and Kookana, 2007). Regarding NPs removal, contradictory results have been reported, possibly due to their formation from alkylphenol ethoxylates during the activated sludge process (Langford et al., 2005). In an early study, Ahel et al. (1994) reported that elimination of the sum of NP1EO and NP2EO ranged from −19% (net formation) to 80%, while NP had an elimination efficiency ranging from 9% to 94%. Gonzalez et al. (2007) reported an average elimination for NP1EO, NP2EO and NP of 46%, 54% and 96%, respectively. On the other hand, Planas et al. (2002) reported NP, NP1EO and NP2EO removal higher than 98% during wastewater treatment.

The fate of EDCs in wastewater treatment process is shown in Fig. 3. Regarding TCS, all loss mechanisms combined (transformation to other metabolites, mineralization to CO<sub>2</sub>) accounted for 46 ± 25% of the total TCS mass input. An equivalent part of TCS (45 ± 27%) was accumulated in sewage sludge, whereas a minor part (9 ± 6%) was removed via the effluents. The findings of this study were almost identical to a previous study of Heidler and Halden (2007), confirming the fate of TCS in STPs using similar treatment processes. Moreover, Bester (2003), investigating the fate of TCS in a German STP, reported that 22–43% of initial TCS was sorbed to the sludge, while about 5% was emitted via the effluents. Regarding the other EDCs, almost 70% of their mass entering the plant was transformed, while fractions of EDCs that were detected in the effluents or sewage sludge were significantly lower (Fig. 3). Similarly, Fauser et al. (2003) reported that 80% of NP2EO was biodegraded, while 2% was found in the treated water and 18% was found in the sludge. The aforementioned results indicate that a significant part of target EDCs is removed during sewage treatment. However, further research is needed to determine the factors that enhance EDCs

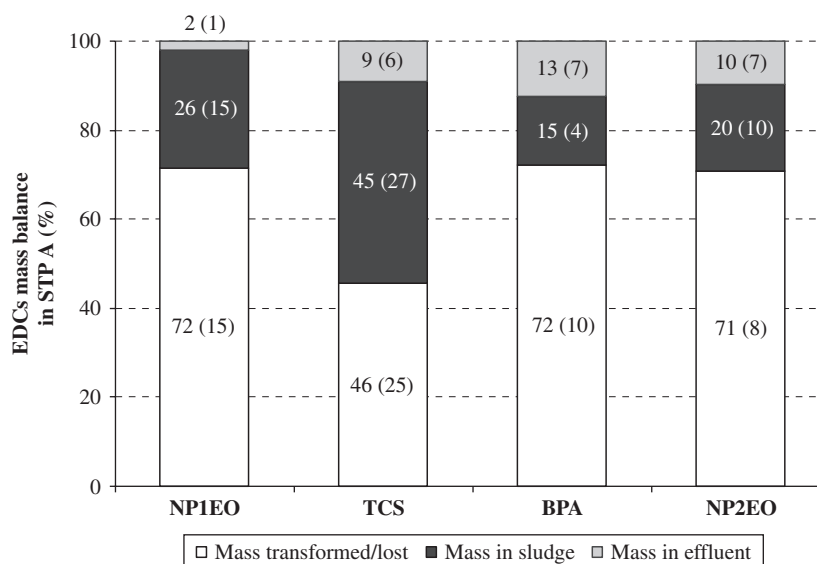


Fig. 3 – EDCs' mass balance in sewage treatment plant A (standard deviations are given in parentheses).

elimination, as well as to investigate possible metabolites formed during biological treatment.

#### 4. Conclusions

Concentrations of EDCs in Greek STPs were generally similar to those that have been previously reported. The highest detected concentrations in influent, treated wastewater and sewage sludge were 23.9 (TCS), 17.4 (NP2EO) and 41.3  $\mu\text{g g}^{-1}$  dw (NP1EO), respectively. In influent wastewater, almost half of these compounds were accumulated in the particulate phase, while a lower fraction (18–35%) was determined in effluents. Concentrations in influent wastewater were significantly lower than those that seem to affect the activated sludge process. However, the results of a preliminary risk assessment showed that potentially adverse effects on aquatic and terrestrial environment should not be excluded. Primary and secondary treatment resulted in a substantial removal of NP1EO, NP2EO, TCS and BPA (removal efficiency >85%). Calculation of mass fluxes showed that degradation and sorption in sewage sludge (especially for TCS) are the main mechanisms affecting EDCs' fate in STPs.

#### Acknowledgments

This study was co-funded by the European Social Fund (75%) and National Resources-(EPEAEK-II) PYTHAGORAS I (25%).

#### Appendix A. Supporting Information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.watres.2007.11.003.

#### REFERENCES

- Ahel, M., Giger, W., Koch, M., 1994. Behaviour of alkylphenol polyethoxylate surfactants in the aquatic environment—I. Occurrence and transformation in sewage treatment. *Water Res.* 28, 1131–1142.
- Bester, K., 2003. Triclosan in a sewage treatment process—balances and monitoring data. *Water Res.* 37, 3891–3896.
- Birkett, J.W., Lester, J.N., 2003. *Endocrine Disrupters in Wastewater and Sludge Treatment Processes*. CRC Press LLC, Florida.
- Bolz, U., Hagenmaier, H., Korner, W., 2001. Phenolic xenoestrogens in surface water, sediments, and sewage sludge from Baden-Wurttemberg south-west Germany. *Environ. Pollut.* 115, 291–301.
- Calafat, A.M., Kuklenyik, Z., Reidy, J.A., Caudill, S.P., Ekong, J., Needham, L.L., 2005. Urinary concentrations of bisphenol A and 4-nonylphenol in a human reference population. *Environ. Health Perspect.* 113, 391–395.
- Chandrinou, S., Stasinakis, A.S., Thomaidis, N.S., Nikolaou, A., Lekkas, T.D., 2007. Distribution of organotin compounds in the bivalves of the Aegean Sea, Greece. *Environ. Int.* 33, 226–232.
- Clara, M., Strenn, B., Gans, O., Martinez, E., Kreuzinger, N., Kroiss, H., 2005. Removal of selected pharmaceuticals, fragrances and endocrine disrupting compounds in a membrane bio-reactor and conventional wastewater treatment plants. *Water Res.* 39, 4797–4807.
- Environment Canada, 2000. Priority Substances List Assessment Report: Nonylphenols and its Ethoxylates. Draft Public Comments, Environment Canada/Health, Canada.
- European Union, 2000. Working document on sludge, Third Draft, European Union, Brussels, Belgium, April 27.
- European Union, Decision no. 2455/2001/EC of the European Parliament and of the council of 20 November 2001 establishing the list of priority substances in the field of water policy and amending directive 2000/60/EC, Off. J. L331, 15/12/2001.
- European Union, 2002. 4-Nonylphenol (branched) and nonylphenol, European Union Risk Assessment Report, EUR 20387 EN. Available from <<http://ecb.jrc.it/DOCUMENTS/Existing-Chemicals/>> (last accessed 2 June 2007).



- Fausser, P., Vikelsoe, J., Sorensen, P.B., Carlsen, L., 2003. Phthalates, nonylphenols and LAS in an alternately operated wastewater treatment plant—fate modelling based on measured concentrations in wastewater and sludge. *Water Res.* 37, 1288–1295.
- Federle, T.W., Kaiser, S.K., Nuck, B.A., 2002. Fate and effects of triclosan in activated sludge. *Environ. Toxicol. Chem.* 21, 1330–1337.
- Fenner, K., Kooijman, C., Scheringer, M., Hungerbuehler, K., 2002. Including transformation products into the risk assessment for chemicals: the case of nonylphenol ethoxylate usage in Switzerland. *Environ. Sci. Technol.* 36, 1147–1154.
- Fent, G., Hein, W.J., Moendel, M., Kubiak, R., 2003. Fate of <sup>14</sup>C-bisphenol A in soils. *Chemosphere* 51, 735–746.
- Fromme, H., Kuchler, T., Otto, T., Pilz, K., Muller, J., Wenzel, A., 2002. Occurrence of phthalates and bisphenol A and F in the environment. *Water Res.* 36, 1429–1438.
- Gatidou, G., Thomaidis, N.S., Stasinakis, A.S., Lekkas, T.D., 2007. Simultaneous determination of the endocrine disrupting compounds nonylphenol, nonylphenol ethoxylates, triclosan and bisphenol A in wastewater and sewage sludge by gas chromatography-mass spectrometry. *J. Chromatogr. A* 1138, 32–41.
- Gomez, M.J., Martinez Bueno, M.J., Lacorte, S., Fernandez-Alba, A.R., Aguera, A., 2007. Pilot survey monitoring pharmaceuticals and related compounds in a sewage treatment plant located on the Mediterranean coast. *Chemosphere* 66, 993–1002.
- Gonzalez, S., Petrovic, M., Barcelo, D., 2007. Removal of a broad range of surfactants from municipal wastewater—comparison between membrane bioreactor and conventional activated sludge treatment. *Chemosphere* 67, 335–343.
- Halden, R.U., Paull, D.H., 2005. Co-occurrence of triclocarban and triclosan in US water resources. *Environ. Sci. Technol.* 39, 1420–1426.
- Heidler, J., Halden, R.U., 2007. Mass balance of triclosan removal during conventional sewage treatment. *Chemosphere* 66, 362–369.
- Isobe, T., Nishiyama, H., Nakashima, A., Takada, H., 2001. Distribution and behavior of nonylphenol, octylphenol, and nonylphenol monoethoxylate in Tokyo Metropolitan Area: their association with aquatic particles and sedimentary distributions. *Environ. Sci. Technol.* 35, 1041–1049.
- Jacobs, M.N., Nolan, G.T., Hood, S.R., 2005. Lignans, bacteriocides and organochlorine compounds activate the human pregnane X receptor (PXR). *Toxicol. Appl. Pharmacol.* 209, 123–133.
- Langford, K.H., Scrimshaw, M.D., Birkett, J.W., Lester, J.N., 2005. Degradation of nonylphenolic surfactants in activated sludge batch tests. *Water Res.* 39, 870–876.
- Lindberg, R.H., Bjorklund, K., Rendahl, P., Johansson, M.I., Tysklind, M., Andersson, B.A.V., 2007. Environmental risk assessment of antibiotics in the Swedish environment with emphasis on sewage treatment plants. *Water Res.* 41, 613–619.
- Mc Avoy, D., Schatowitz, B., Jacob, M., Hauk, A., Eckhoff, W.S., 2002. Measurement of triclosan in wastewater treatment systems. *Environ. Toxicol. Chem.* 21, 1323–1329.
- Nakada, N., Tanishima, T., Shinohara, H., Kiri, K., Takada, H., 2006. Pharmaceutical chemicals and endocrine disruptors in municipal wastewater in Tokyo and their removal during activated sludge treatment. *Water Res.* 40, 3297–3303.
- Patrolecco, L., Capri, S., De Angelis, S., Pagnotta, R., Polesello, S., Valsecchi, S., 2006. Partition of nonylphenol and related compounds among different aquatic compartments in Tiber river (Central Italy). *Water Air Soil Pollut.* 172, 151–166.
- Planas, C., Guadayol, J.M., Droguet, M., Escalas, A., Rivera, J., Caixach, J., 2002. Degradation of polyethoxylated nonylphenols in a sewage treatment plant. Quantitative analysis by isotopic dilution-HRGC/MS. *Water Res.* 36, 982–988.
- Roberts, P., Roberts, J.P., Jones, D.L., 2006. Behaviour of the endocrine disrupting chemical nonylphenol in soil: assessing the risk associated with spreading contaminated waste to land. *Soil Biol. Biochem.* 38, 1812–1822.
- Singer, H., Muller, S., Tixier, C., Pillonel, L., 2002. Triclosan: occurrence and fate of a widely used biocide in the aquatic environment: field measurements in wastewater treatment plants, surface waters, and lake sediments. *Environ. Sci. Technol.* 36, 4998–5004.
- Stasinakis, A.S., Mamais, D., Thomaidis, N.S., Danika, E., Gatidou, G., Lekkas, T.D., 2006. Investigation of triclosan and nonylphenol acute toxicity in activated sludge process and aquatic environment. SETAC Europe 16th Annual Meeting, May 7–11, 2006, Hague, The Netherlands, p. 152.
- Stasinakis, A.S., Petalas, A.V., Mamais, D., Thomaidis, N.S., Gatidou, G., Lekkas, T.D., 2007. Investigation of triclosan fate and toxicity in continuous-flow activated sludge systems. *Chemosphere* 68, 375–381.
- Vetillard, A., Bailhache, T., 2006. Effects of 4-n-nonylphenol and tamoxifen on salmon gonadotropin-releasing hormone, estrogen receptor, and vitellogenin gene expression in juvenile rainbow trout. *Toxicol. Sci.* 92, 537–544.
- Vogelsang, C., Grung, M., Jantsch, T.G., Tollefsen, K.E., Litved, H., 2006. Occurrence and removal of selected organic micropollutants at mechanical, chemical and advanced wastewater treatment plants in Norway. *Water Res.* 40, 3559–3570.
- Waltman, E.L., Barney, J.V., Waller, W.T., 2006. Triclosan in a North Texas wastewater treatment plant and the influent and effluent of an experimental constructed wetland. *Environ. Toxicol. Chem.* 25, 367–372.
- Ying, G.G., Kookana, R.S., 2007. Triclosan in wastewaters and biosolids from Australian wastewater treatment plants. *Environ. Int.* 33, 199–205.
- Ying, G.G., Kookana, R.S., Dillon, P., 2003. Sorption and degradation of selected five endocrine disrupting chemicals in aquifer material. *Water Res.* 37, 3785–3791.